

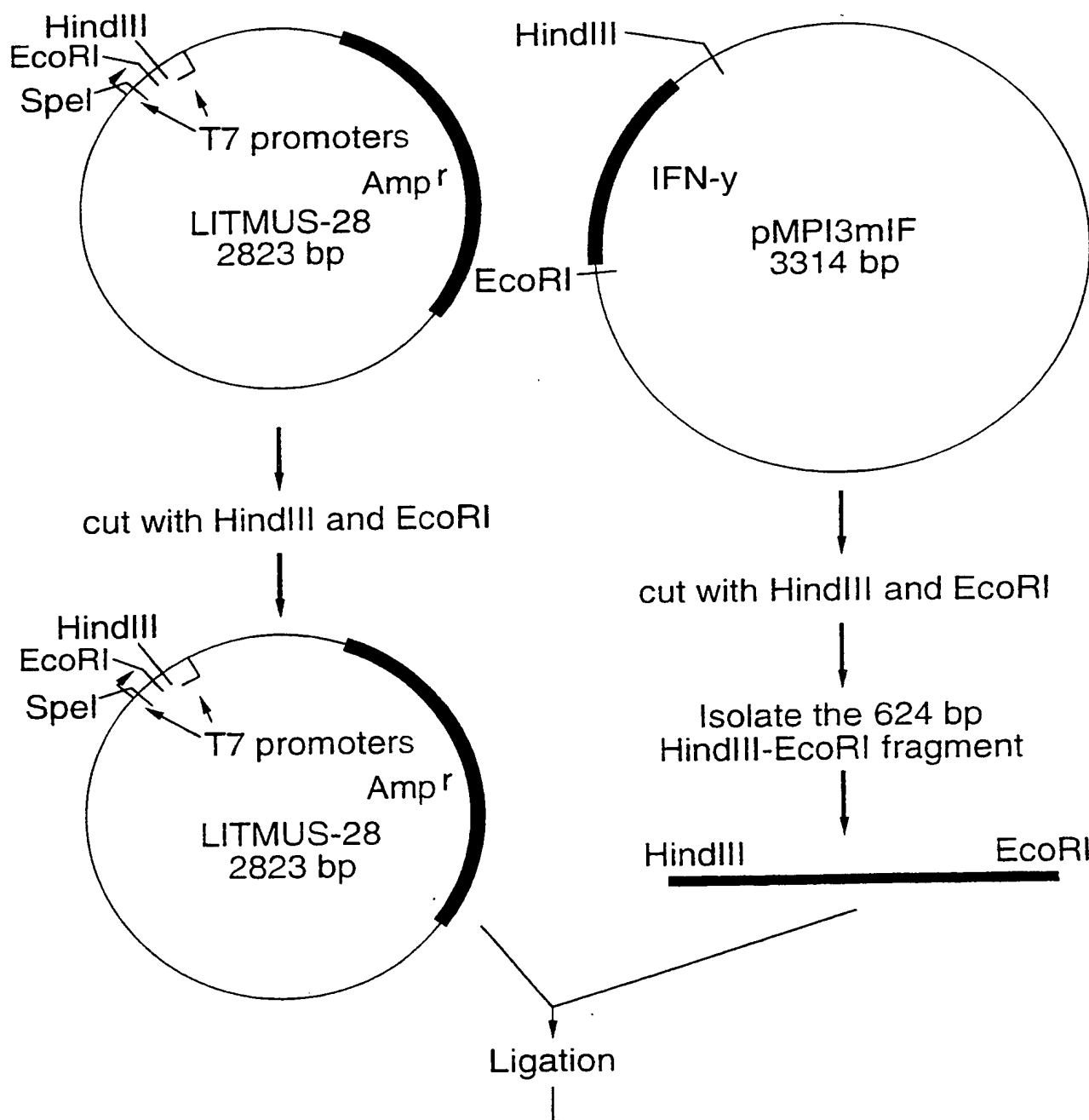
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Construction of pLITMUS-IFN- $\gamma$ 

FIG.1A

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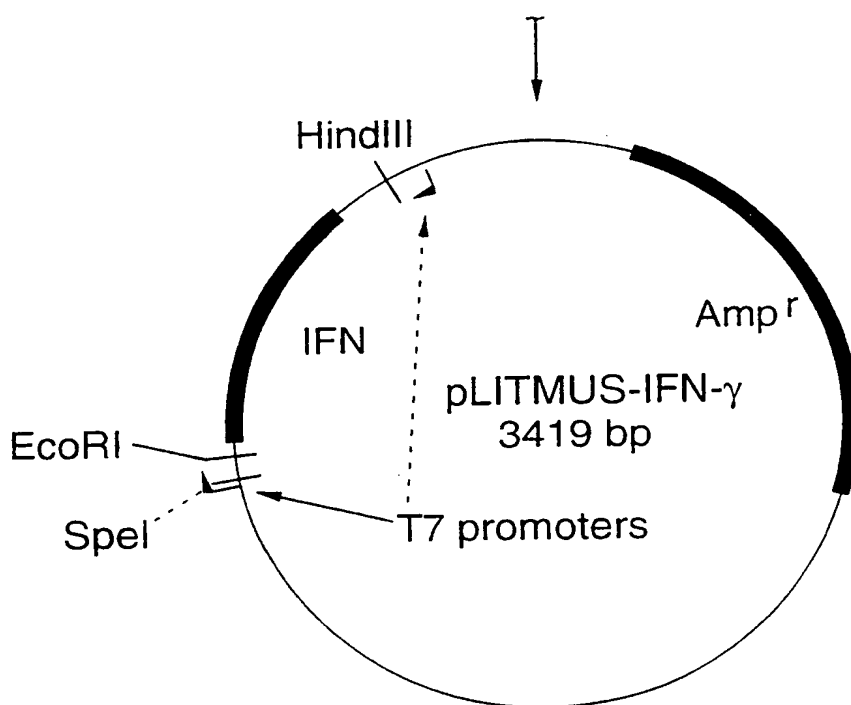


FIG.1B

FIG.2A

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Sequence of pLITMUS-IFN- $\gamma$ 

GTAACTACG TCAGGTGGCA CTTTTCGGGG AAATGTGCGC GGAACCCCTA TTGTTTTATT  
TTTCTAAATA CATTCAAATA TGTATCCGCT CATGAGACAA TAACCCTGAT AAATGCTTCA  
ATAATATTGA AAAAGGAAGA GTATGAGTAT TCAACATTTC CGTGTGCGCC TTATTCCCTT  
TTTTGCGGCA TTTTGCCCTC CTGTTTTTGC TCACCCAGAA ACGCTGGTGA AAGTAAAAGA  
TGCTGAAGAT CAGTTGGGTG CACGAGTGGG TTACATCGAA CTGGATCTCA ACAGCGGTAA  
GATCCTTGAG AGTTTTCGCC CCGAAGAACG TTCTCCAATG ATGAGCACTT TTAAAGTTCT  
GCTATGTGGC GCGGTATTAT CCGTGTGTA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT  
ACACTATTCT CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC ATCTTACGGA  
TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC ATGAGTGATA ACGTGGCGC  
CAACTTACTT CTGACAACGA TCGGAGGACC GAAGGAGCTA ACCGCTTTTT TGCACAACAT  
GGGGGATCAT GTAACGCGC TTGATCGTTG GGAACCGGAG CTGAATGAAG CCATACCAAA  
CGACGAGCGT GACACCACGA TGCCGTGTAGC AATGGCAACA ACGTTGCGCA AACTATTAAAC  
TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA GACTGGATGG AGGCGGATAA  
AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC TGGTTTTATT CTGATAAATC  
TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT CATTGCAGCA CTGGGGCCAG ATGGTAAGCC  
CTCCCGTATC GTAGTTATCT ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG  
ACAGATCGCT GAGATAGGTG CCTCACTGAT TAAGCATTGG TAACTGTCAG ACCAAGTTTA  
CTCATATATA CTTTAGATTG ATTTACCCCG GTTGATAATC AGAAAAGCCC CAAAAACAGG  
AAGATTGTAT AAGCAAATAT TTAAATTGTA AACGTTAATA TTTTGTAAAT ATTTCGCGTTA  
AATTTTTGTT AAATCAGCTC ATTTTTTAAC CAATAGGCCG AAATCGGCAA AATCCCTTAT  
AAATCAAAAG AATAGCCCGA GATAGGGTGT AGTGTGTGTT CAGTTTGGAA CAAGAGTCCA  
CTATTAAAGA ACGTGGACTC CAACGTCAA GGGCGAAAA CCGTCTATCA GGGCGATGGC  
CCACTACGTG AACCATCACC CAAATCAAGT TTTTTGGGGT CGAGGTGCCG TAAAGCACTA  
AATCGGAACC CTAAAGGGAG CCCCCGATTT AGAGCTTGAC GGGGAAAGCG AACGTGGCGA  
GAAAGGAAGG GAAGAAAGCG AAAGGAGCGG GCGCTAGGGC GCTGGCAAGT GTAGCGGTCA  
CGCTGCGCGT AACCACCACA CCGCGCGCGC TTAATGCGCC GCTACAGGGC GCGTAAAAGG  
ATCTAGGTGA AGATCCTTTT TGATAATCTC ATGACCAAAA TCCCTTAACG TGAGTTTTTCG  
TTCCACTGAG CGTCAGACCC CGTAGAAAAG ATCAAAGGAT CTTCTTGAGA TCCTTTTTTTT  
CTGCGCGTAA TCTGCTGCTT GCAAACAAAA AAACCACCGC TACCAGCGGT GGTTTGTTTG  
CCGGATCAAG AGCTACCAAC TCTTTTTTCCG AAGGTAAGT GCTTCAGCAG AGCGCAGATA  
CCAAATACTG TTCTTCTAGT GTAGCCGTAG TTAGGCCACC ACTTCAAGAA CTCTGTAGCA  
CCGCCTACAT ACCTCGCTCT GCTAATCCTG TTACCAGTGG CTGCTGCCAG TGGCGATAAG  
TCGTGTCTTA CCGGGTTGGA CTCAAGACGA TAGTTACCGG ATAAGGCGCA GCGGTGCGGC  
TGAACGGGGG GTTCGTGCAC ACAGCCCAGC TTGGAGCGAA CGACCTACAC CGAACTGAGA  
TACCTACAGC GTGAGCTATG AGAAAGCGCC ACGCTTCCCG AAGGGAGAAA GGCGGACAGG  
TATCCGGTAA GCGGCAGGGT CGGAACAGGA GAGCGCACGA GGGAGCTTCC AGGGGGAAAC

FIG.2B

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GCCTGGTATC TTTATAGTCC TGTCGGGTTT CGCCACCTCT GACTTGAGCG TCGATTTTTCG  
TGATGCTCGT CAGGGGGGCG GAGCCTATGG AAAAACGCCA GCAACGCGGC CTTTTTACGG  
TTCCTGGCCT TTTGCTGGCC TTTTGCTCAC ATGTAATGTG AGTTAGCTCA CTCATTAGGC  
ACCCCAGGCT TTACACTTTA TGCTTCCGGC TCGTATGTTG TGTGGAATTG TGAGCGGATA  
ACAATTTTAC ACAGGAAACA GCTATGACCA TGATTACGCC AAGCTACGTA ATACGACTCA  
CTAGTGGGCA GATCTTCGAA TGCATCGCGC GCACCGTACG TCTCGAGGAA TTCCCGGGGA  
TCCCTGCAGT TATTGGGACA ATCTCTTCCC CACCCCGAAT CAGCAGCGAC TCCTTTTCCG  
CTTCTGAGG CTGGATTCCG GCAACAGCTG GTGGACCACT CGGATGAGCT CATTGAATGC  
TTGGCGCTGG ACCTGTGGGT TGTTGACCTC AAACCTGGCA ATACTCATGA ATGCATCCTT  
TTTCGCCCTG CTGTTGCTGA AGAAGGTAGT AATCAGGTGT GATTCAATGA CGCTTATGTT  
GTTGCTGATG GCCTGATTGT CTTTCAAGAC TTCAAAGAGT CTGAGGTAGA AAGAGATAAT  
CTGGCTCTGC AGGATTTTCA TGTCACCATC CTTTTGCCAG TTCCTCCAGA TATCCAAGAA  
GAGACTCTTT TCTTCCACAT CTATGCCACT TGAGTTAAAA TAGTTATTCA GACTTCTAG  
GCTTTCAATG ACTGTGCCGT GGCAGTAACA GCCAGAAACA GCCATGAGGA AGAGCTGCAA  
AGCCAAGATG CAGTGTGTAG CGTTCATGAT TAGATTAAAC TAAATAATTG TACTTTGTAA  
TATAATGATA TATATTTTCA CTTTATCTCA TTTGAGAATA AAAATGTTTT TGTTTAACCA  
CTGCATGATG TAAGCTTCCC ATGGTGACGT CACCGGTTCT AGATACCTAG GTGAGCTCTG  
GTACCCCTCTA GTCAAGGCCT TAAGTGAGTC GTATTACGGA CTGGCCGTCG TTTTACAACG  
TCGTGACTGG GAAAACCTG GCGTTACCCA ACTTAATCGC CTTGCAGCAC ATCCCCCTTT  
CGCCAGCTGG CGTAATAGCG AAGAGGCCCG CACCGATCGC CCTTCCCAAC AGTTGCGCAG  
CCTGAATGGC GAATGGCGCT TCGCTTGGTA ATAAAGCCCG CTTCGGCGGG CTTTTTTTTT

Utilized T7 promoter 3199 - 3216

Transcription start site 3198

IFN-( specific sequence 2530 - 3027

Transcript sequence 2464 - 3198

SpeI site used for linearization 2461

## FIG.3A

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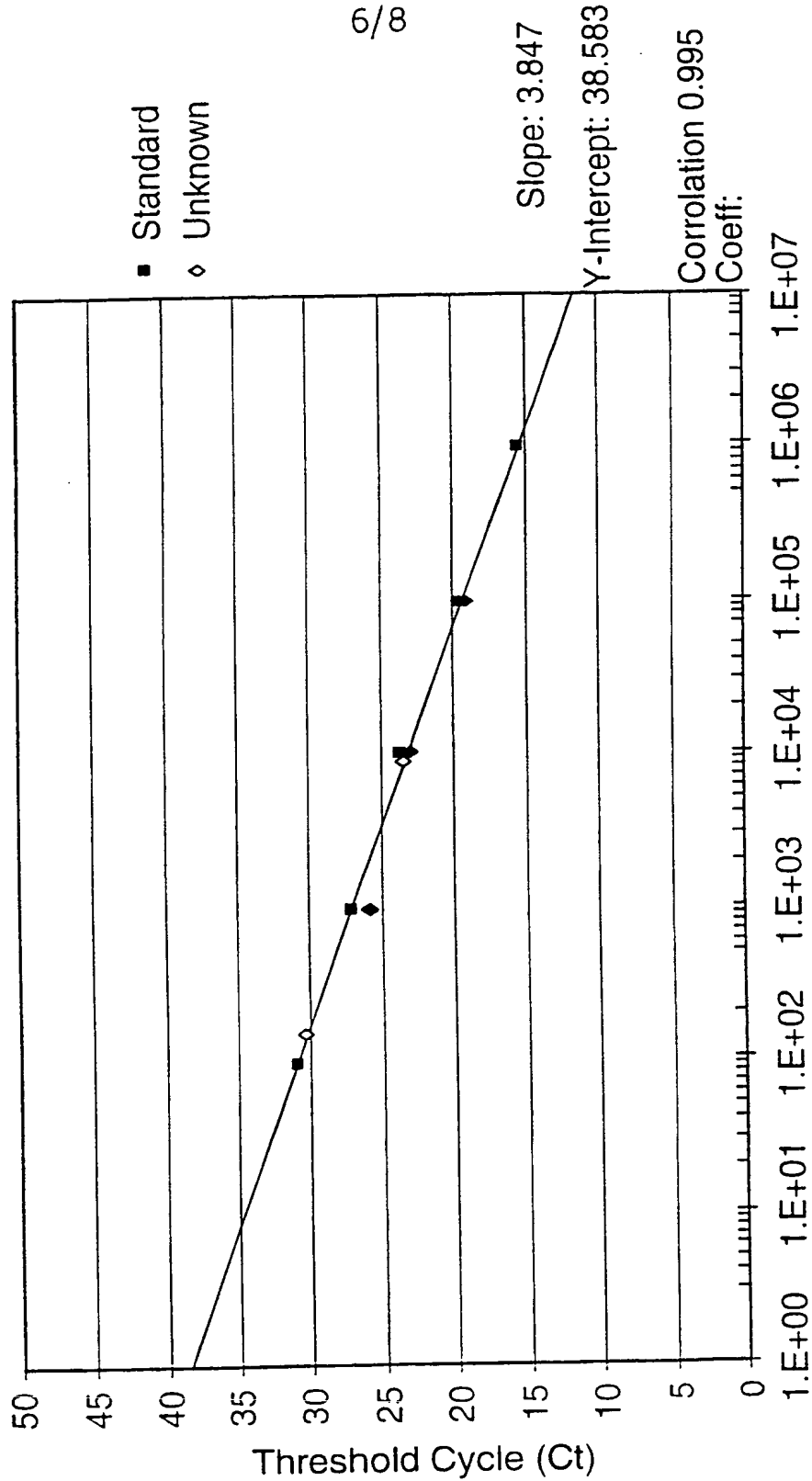
Sequence of the IFN- $\gamma$  RNA Standard

1 GCCUUGACUA GAGGGUACCA GAGCUCACCU AGGUAUCUAG AACCGGUGAC 50  
51 GUCACCAUGG GAAGCUUACA UCAUGCAGUG GUUAAACAAA AACAUUUUUA 100  
101 UUCUCAAAUG AGAUAAAGUG AAAAUUAUUA UCAUUAUUAU ACAAGUACA 150  
151 AUUAUUUAGG UAAAUCAAU CAUGAACGCU ACACACUGCA UCUUGGCUUU 200  
201 GCAGCUCUUC CUCAUGGCUG UUUCUGGCUG UUACUGCCAC GGCACAGUCA 250  
251 UUGAAAGCCU AGAAAGUCUG AAUAACUAU UUAACUCAAG UGGCAUAGAU 300  
301 GUGGAAGAAA AGAGUCUCUU CUUGGAUAUC UGGAGGAACU GGCAAAAGGA 350  
351 UGGUGACAUG AAAAUCCUGC AGAGCCAGAU UAUCUCUUUC UACCUCAGAC 400  
401 UCUUUGAAGU CUUGAAAGAC AAUCAGGCCA UCAGCAACAA CAUAAGCGUC 450  
451 AUUGAAUCAC ACCUGAUUAC UACCUUCUUC AGCAACAGCA AGGCGAAAAA 500  
501 GGAUGCAUUC AUGAGUAUUG CCAAGUUUGA GGUCAACAAC CCACAGGUCC 550  
551 AGCGCCAAGC AUUCA AUGAG CUCAUCCGAG UGGUCCACCA GCUGUUGCCG 600  
601 GAAUCCAGCC UCAGGAAGCG GAAAAGGAGU CGCUGCUGAU UCGGGGUGGG 650  
651 GAAGAGAUUG UCCCAAUAAC UGCAGGGAUC CCCGGGAUU CCUCGAGACG 700  
701 UACGGUGCGC GCGAUGCAUU CGAAGAUUC CCCACUAG 738

IFN- $\gamma$  specific sequence 172 - 669

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Standard Curve: JC RT-PCR 92  $\gamma$ -IFN



Starting Quantity

FIG.4

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RT-PCR Cycle Optimization for Lialuid Hybridization

PCR Cycle Optimization for IL5 Amplification

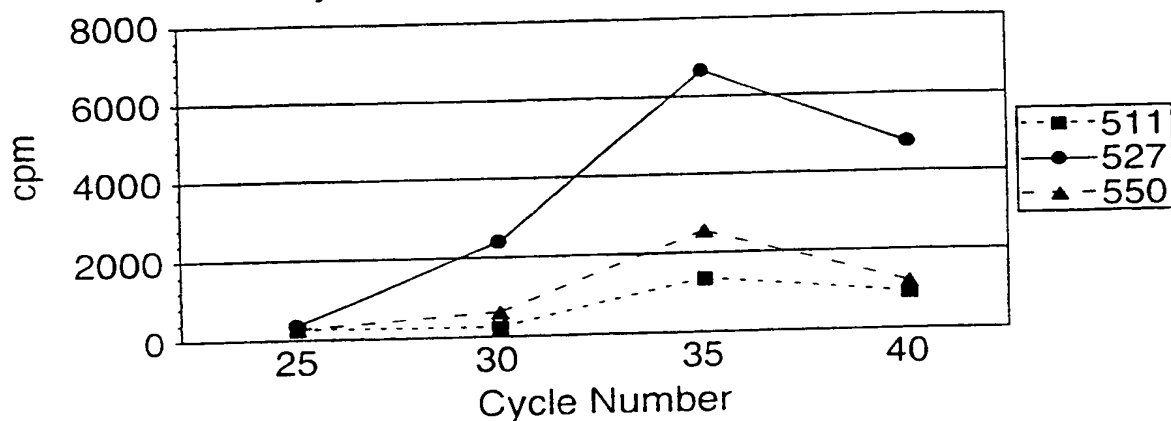


FIG.5A

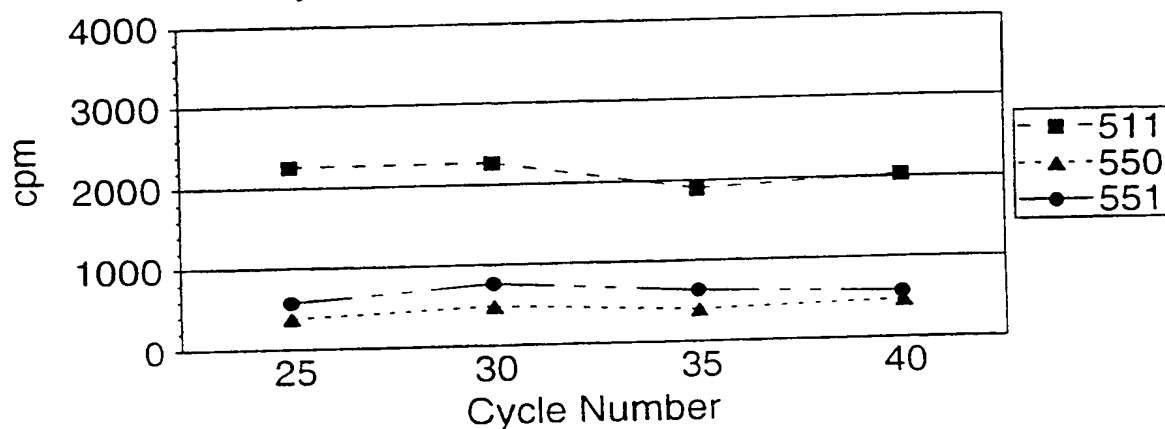
PCR Cycle Optimization for  $\gamma$ -IFN Amplification

FIG.5B

PCR Cycle Optimization for IL4 Amplification

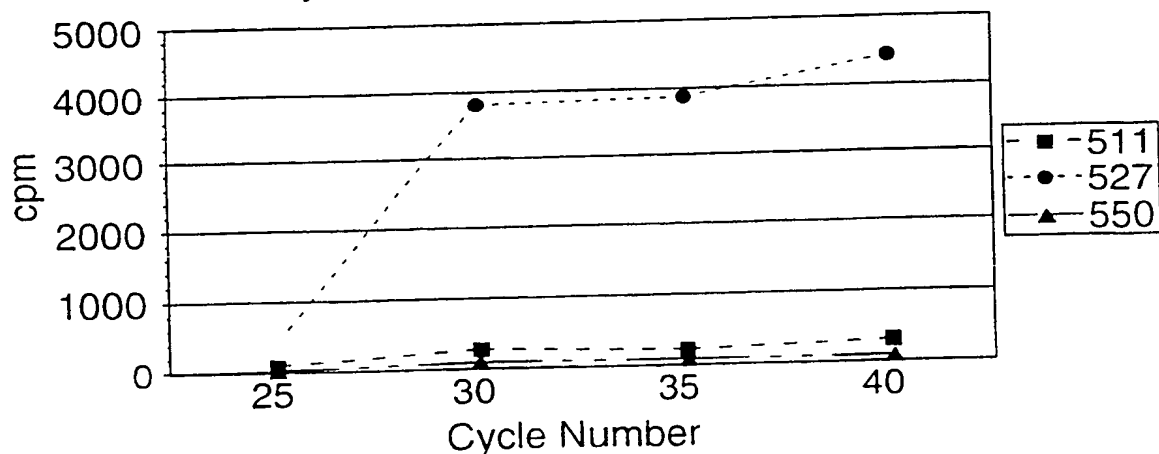


FIG.5C

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